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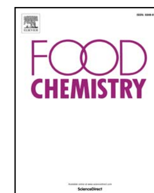

Exploring reserve lots of *Cymbopogon citratus*, *Aloysia citrodora* and *Thymus* × *citriodorus* as improved sources of phenolic compounds

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A B S T R A C T

Given the increasing consumers demand for novelty, tea companies have been presenting new added value products such as reserve lots of aromatic plants. Herein, infusions from different lots of three aromatic plants were assessed in terms of phenolic composition (HPLC-DAD-ESI/MS) and antioxidant properties (reducing power, free radical scavenging and lipid peroxidation inhibition capacity). *Cymbopogon citratus* (*C. citratus*; main compound 5-*O*-caffeoylquinic acid) and *Aloysia citrodora* (*A. citrodora*; prevalence of verbascoside) reserve lots revealed higher phenolic compounds concentration than the respective standard lots. *Thymus* × *citriodorus* (*T. citriodorus*; main compound rosmarinic acid) standard lot presented higher amounts of phenolic acids than the reserve lot, nonetheless, total flavonoids and phenolic compounds were not significantly different. The differences between both lots antioxidant activity were more noticeable in *C. citratus*, with the reserve lot presenting the highest activity. This study provides evidence of the differences between these plants chemical composition and bioactivity depending on the harvesting conditions.

1. Introduction

Over the past few years, an increasing number of consumers are looking for new food products with distinctive features that provide pleasant moments while affording health benefits (Asioli et al., 2017; McCarthy & Liu, 2017; Román, Sánchez-Siles, & Siegrist, 2017; Yasmeen, Fukagawa, & Wang, 2017). Such products include aromatic plants infusions, which represented, in 2015, a consumption of 5.2 million tons worldwide (Statista, 2015). In Europe, more than 400 different parts of plants are used for the preparation of infusions with a wide range of flavours and, simultaneously, varied health benefits (Schulzki, Nüβlein, & Sievers, 2017). Owing to this increasing consumption level, and in an attempt to meet consumers' requirements, tea companies have been introducing new added-value products with different characteristics (Gramza-Michałowska, Kulczyński, Xindi, & Gumienna, 2016). The so-called reserve lots, for example, are prepared through distinct harvesting techniques that differentiate them from standard lots, not only in terms of sensorial characteristics, but also regarding their chemical composition (Rita, Pereira, Barros, Santos-Buelga, & Ferreira, 2016).

Phenolic compounds are an example of plants' secondary metabolites that are affected by harvest conditions, mainly the maturation state, and are considered the most bioactive compounds of medicinal plants infusions and decoctions (Barroso et al., 2016; Costa, Grangeia, Figueirinha, Figueiredo, & Batista, 2016; da Silveira, Meinhart, Ballus, & Godoy, 2014; Fotakis et al., 2016).

Among the most consumed specimens, *Cymbopogon citratus* (DC.) Stapf (*C. citratus*), *Aloysia citrodora* (L'Herit.) Britton (*A. citrodora*), and *Thymus* × *citriodorus* L. (*T. citriodorus*) have been widely used for their pleasant taste and health promoting properties (Bensabah, Lamiri, & Naja, 2015; Coelho et al., 2016; Pereira, Peres, Silva, Domingues, & Cardoso, 2013). *C. citratus*, belonging to the Poaceae (Graminae) family, and commonly known as lemongrass, is an Indian and tropical Asia endemic crop that, nowadays, grows worldwide. It has a significant economic value for its diverse industrial applications, such as perfumery, cosmetic, pharmaceutical and food industry (Costa et al., 2016). In herbal medicine, it is used as antibacterial, antifungal, antiprotozoal, anticancer, anti-inflammatory, antioxidant, cardioprotective, antitussive, antiseptic, and antirheumatic (Ajayi, Sadimenko, & Afolayan, 2016; Chukwuocha, Fernández-Rivera, & Legorreta-Herrera, 2016; Ekpenyong, Akpan, & Nyoh, 2015). It has also been used in the prevention of platelet aggregation in the treatment of diabetes, dyslipidemia, gastrointestinal disorders, anxiety, malaria, flu, fever and pneumonia (Costa et al., 2016).

On the other hand, *A. citrodora* is included in the Verbenaceae family and is commonly known as lemon verbena. It is native from South America and was introduced into Europe in the end of the 17th century, being today cultivated in the Mediterranean and North Africa

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(Marocco) (Bensabah et al., 2015; dos Santos et al., 2017). Its leaves infusion has been widely used in folk medicine for colds, fever, flatulence, colic, diarrhoea, spasms asthma, anxiety, insomnia, and indigestion cases (Carnat, Carnat, Fraisse, & Lamaison, 1999; dos Santos et al., 2017). Besides these therapeutic characteristics, its leaves are widely appreciated for their characteristic lemony scent, being therefore used in many food preparations, such as fish and poultry dishes, vegetable marinades, salad dressings, jams, puddings, beverages, and sorbets (Funes et al., 2009).

Lastly, *T. citriodorus*, also known as lemon thyme, belongs to the Lamiaceae family. It is native from Southern Europe and cultivated in Mediterranean area for culinary purposes, due to its pleasant lemon flavour. It has traditionally been used for bronchitis cases and as diaphoretic, but also possesses antimicrobial and antifungal activity (Omidbaigi, Sefidkon, & Hejazi, 2005).

Given these plants infusions wide consumption, the present study aimed to deepen the scientific knowledge regarding the antioxidant activity and phenolic profile variation between standard and reserve lots.

2. Materials and methods

2.1. Samples and samples preparation

The dry material of the three species (*A. citrodora*, *T. citriodorus*, and *C. citratus*) used for infusions preparation, both standard and reserve lots, was provided by *Cantinho das Aromáticas* (organic farmers from Vila Nova de Gaia, Portugal). According to the information provided by the producers, reserve lots were prepared through different harvest procedures, in the hot summer months, being composed by the younger parts (apical leaves) of the species (Rita et al. 2016).

All the infusions were prepared according to the specifications mentioned on the label, except for *C. citratus*, which infusions were prepared in higher concentrations in order to allow the analyses. The infusions of *A. citrodora*, *T. citriodorus*, and *C. citratus* were obtained as follows: 100 mL of heated distilled water (at 90, 85, and 90 °C, respectively) were added to dry plant material (300, 300, and 900 mg, respectively) and the infusions were left to stand (for 7, 7, and 5 min, respectively) until filtration. The obtained infusions were used to perform the different antioxidant activity assays (preparing further dilutions from the stock solution) and to analyse the phenolic profile (after filtration through 0.2 µm nylon filters; Whatman™, Maidstone, UK). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.2. Analysis of phenolic compounds

The filtered infusions were submitted to HPLC-DAD-ESI/MS analysis. Chromatographic data were acquired from Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) following a procedure previously reported (Bessada, Barreira, Barros, Ferreira, & Oliveira, 2016). This system consists of a diode array detector coupled to an electrospray ionization mass detector (LC-DAD-ESI/MSn). Waters Spherisorb S3 ODS-2 C_{18} column (3 µm, 4.6 × 150 mm, Waters, Milford, MA, USA) allowed chromatographic separation and the gradient used consisted of the following solvents (A) 0.1% formic acid (LAB-SCAN analytical sciences, Gliwice, Poland) in water, and (B) acetonitrile (LAB-SCAN analytical sciences, Gliwice, Poland). The gradient elution applied was: 15% B (0–5 min), 15%–20% B (5–10 min), 20–25% B (10–20 min), 25–35% B (20–30 min), and 35–50% B (30–40 min); the column was then re-equilibrated using a flow rate of 0.5 mL/min. Data were collected simultaneously with a DAD (280, 330 and 370 nm) and in a mass spectrometer. Negative mode was chosen for MS detection on a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA). Sheath gas (nitrogen) was kept on 50 psi. Other parameters settings: 325 °C of source temperature, 5 kV of spray voltage, −20 V of capillary voltage, −66 V of tube lens offset, and 35 arbitrary units of collision energy. The full scan captured the mass between *m/z* 100 and 1500. Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA) was used for data acquisition.

For the identification of phenolic compounds, retention times, UV–VIS and mass spectra were compared with available standards (apigenin 6-*C*-glucoside ≥ 99%, apigenin 7-*O*-glucoside ≥ 99%, caffeic acid ≥ 99%, chlorogenic acid ≥ 99%, *p*-coumaric acid ≥ 90%; naringenin ≥ 99%, quercetin 3-*O*-glucoside ≥ 99%, rosmarinic acid ≥ 99% HPLC purity, Extrasynthèse, Genay, France). Literature data were used to tentatively identify the remaining compounds. For the quantitative analysis of phenolic compounds, a calibration curve was obtained by injection of known concentrations (2.5–100 g/ml) of different standard compounds. The identified phenolic compounds with unavailable commercial standard were quantified via calibration curve of the most similar standard available. The results were expressed as µg/mL of infusion.

2.3. Evaluation of the antioxidant activity

The antioxidant activity of the infusions prepared from standard and reserve lots was evaluated trough four different assays, DPPH radical-scavenging activity, reducing power, β-carotene bleaching inhibition and lipid peroxidation inhibition by TBARS assay, as previously described by the authors (Rita et al. 2016). The results were converted into EC_{50} values (mg/mL), which represent the infusion concentration that presents the capacity to provide 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma, St. Louis, MO, USA) was used as positive control.

2.4. Statistical analysis

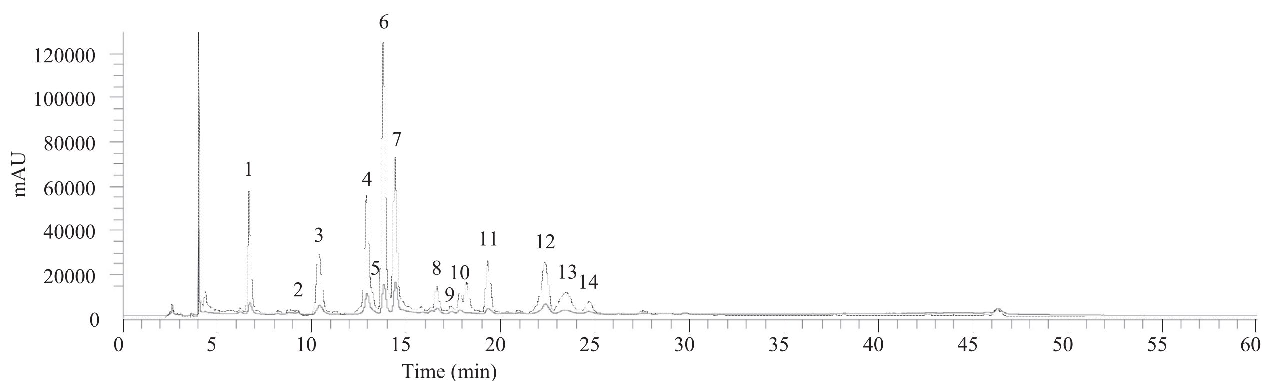
All the assays were carried out using triplicates of the samples with different concentrations and respective spectrophotometric measurements. The results were expressed as mean values and standard deviations (SD) and analysed using a Student's *t*-test in order to determine the significant difference among two different samples, with a *p*-value of 0.05. When the *p*-value was lower than 0.05, significant differences between samples were considered. Furthermore, a Pearson's correlation analysis between the antioxidant activity and all the identified compounds was carried out, with a 95% confidence level. These statistical treatments were carried out using SPSS v. 23.0 program (IBM Corp., Armonk, NY, USA).

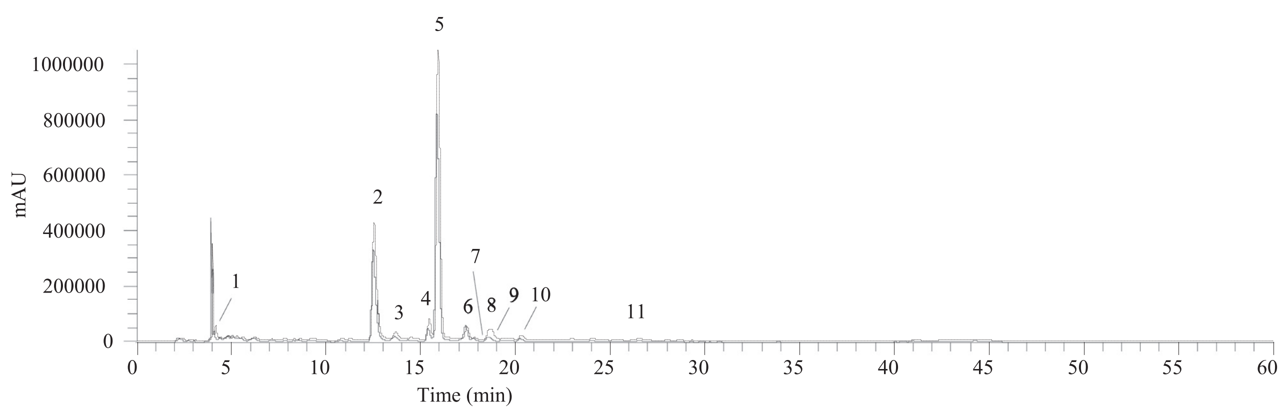
3. Results and discussion

3.1. Phenolic compounds

The identification of individual phenolic compounds was carried out considering their retention times, whenever possible in comparison with commercially available standards, and both UV and MS spectra. The phenolic profile of the samples revealing the highest contents of phenolic compounds is shown in Fig. 1. Data obtained by HPLC-DAD-ESI/MS analysis (retention time, λ_{max}, pseudomolecular ion, main fragment ions in MS^2), phenolic compounds identification, and respective quantification are present in Tables 1–3.

Regarding *C. citratus*, the standard and reserve lots revealed similar phenolic profiles in terms of detected compounds, but the reserve lot presented significantly higher concentrations than the standard lot, with a total amount of 88 and 11.1 µg/mL, respectively. Both lots revealed a prevalence of flavonoids, with the highest contributions of luteolin 2″-*O*-deoxyhexosyl-6-*C*-glucoside and luteolin 6-*C*-pentosyl-8-*C*-pentoside, but the most abundant compound was 5-*O*-caffeoylquinic acid in both lots. All the identified compounds were previously found and described by our research group in *C. citratus* (Roriz, Barros,





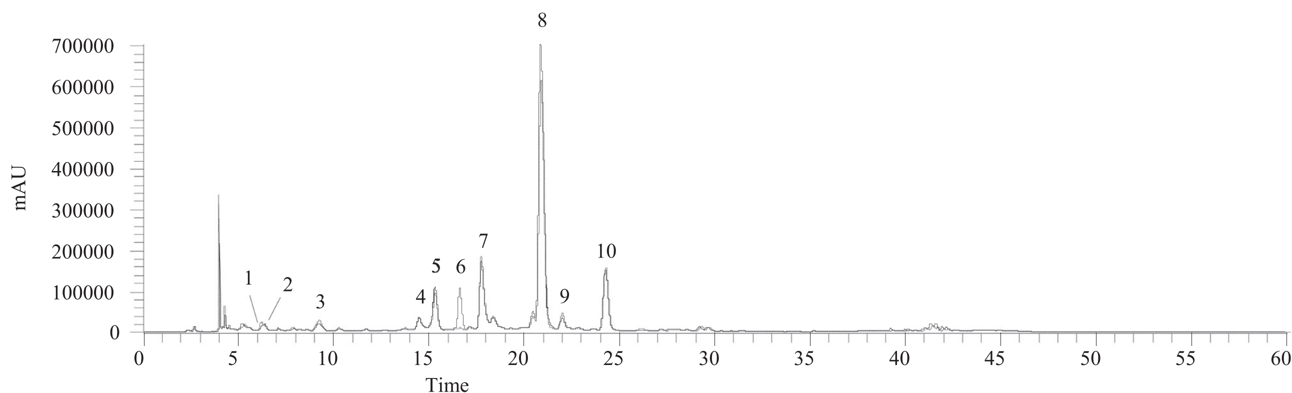


Fig. 1. Phenolic profile of *C. citratus* (recorded at 370 nm; A), *A. citrodora* (recorded at 280 nm; B), and *T. citriodorus* (recorded at 280 nm; C) infusions; dotted line (…) – reserve lot, full line (−) – standard lot. Peak numbering is in accordance with Tables 1–3.

Carvalho, Santos-Buelga, & Ferreira, 2014), where the major flavonoid detected was also luteolin 2″-*O*-deoxyhexosyl-6-*C*-glucoside but, contrarily to the results obtained in the present study, in higher concentrations than 5-*O*-caffeoylquinic acid. This variation could be attributed to the different harvest conditions, given the fact that hydroxycinnamic acids appear among the compounds that present the highest variability over spring-summer months (Costa et al., 2016).

In the case of *A. citrodora*, similar observations could be made, with the reserve lot (230 μg/mL) presenting higher concentrations of phenolic compounds than the standard lot (182 μg/mL). *p*-Coumaric acid was the only phenolic acid detected, in concentrations of 1.65 and 1.91 μg/mL in standard and reserve lots, respectively. Flavonoids and caffeoyl phenylethanoid derivatives were present in similar amounts in both lots, but in slightly higher concentrations in the reserve lot. In a study where we previously assessed the phenolic composition of *A. citrodora* infusions (Pereira, Pimenta, et al., 2017), the amounts of total caffeoyl derivatives (146.0 μg/mL) were higher than in the infusions studied herein (86.1 and 112 μg/mL for standard and reserve lots,

Table 1
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification and quantification of phenolic compounds in *C. citratus* infusions, standard and reserve lots (mean ± SD).

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	Main MS^2 fragments (*m/z*)	Tentative identification	Quantification (μg/mL)		*t*-Students test *p*-value
						Standard	Reserve	
1^{Cc}	6.70	324	353	191(100), 179(4), 161(5), 135(3)	5-*O*-Caffeoylquinic acid	3.87 ± 0.05	23.3 ± 0.2	< 0.001
2^{Cc}	9.23	326	179	135(100)	Caffeic acid	1.13 ± 0.01	2.88 ± 0.03	< 0.001
3^{Cc}	10.38	350	579	561(10), 519(6), 489(56), 459(100), 399(52), 369(47)	Luteolin 6-*C*-hexosyl-8-*C*-pentoside	tr	5.610 ± 0.003	–
4^{Cc}	12.88	336	563	545(27), 503(40), 473(100), 443(83), 383(90), 353(85)	Apigenin 6-*C*-pentosyl-8-*C*-hexoside	1.2 ± 0.1	7.63 ± 0.08	< 0.001
5^{Cc}	12.97	336	563	545(20), 503(37), 473(100), 443(83), 383(73), 353(83)	Apigenin 6-*C*-pentosyl-8-*C*-hexoside	tr	1.1 ± 0.2	< 0.001
6^{Cc}	13.78	350	593	473(100), 429(37), 357(26), 339(12), 309(20), (35)	Luteolin 2″-*O*-deoxyhexosyl-6-*C*-glucoside	1.78 ± 0.09	15.7 ± 0.1	< 0.001
7^{Cc}	14.40	350	549	531(18), 489(36), 459(100), 441(25), 429(21), 399(55), 369(52)	Luteolin 6-*C*-pentosyl-8-*C*-pentoside	2.02 ± 0.07	9.7 ± 0.2	< 0.001
8^{Cc}	16.60	344	577	457(26), 413(100), 341(15), 311(14), 293(77)	Apigenin 2″-*O*-deoxyhexosyl-*C*-hexoside	nd	1.8 ± 0.1	–
9^{Cc}	17.36	352	563	503(3), 473(100), 417(17), 399(53), 357(23), 327(25), 298(40)	Luteolin 2″-*O*-deoxyhexosyl-*C*-pentoside	tr	1.22 ± 0.03	–
10^{Cc}	17.80	350	593	447(6), 285(22)	Luteolin 7-*O*-neohesperoside	tr	2.26 ± 0.05	–
11^{Cc}	19.29	352	563	503(3), 473(100), 417(17), 399(53), 357(23), 327(25), 298(40)	Luteolin 2″-*O*-deoxyhexosyl-*C*-pentoside	tr	4.22 ± 0.02	–
12^{Cc}	22.34	350	575	531(33), 429(38), 411(100), 367(65), 357(15), 337(20), 309(10)	Luteolin 2″-*O*-deoxyosyl-*C*-(6-deoxy-*pento*-hexos-ulosyl)	1.13 ± 0.03	6.52 ± 0.07	< 0.001
13^{Cc}	23.38	350	575	531(33), 429(38), 411(100), 367(65), 357(15), 337(20), 309(10)	Luteolin 2″-*O*-deoxyosyl-*C*-(6-deoxy-*pento*-hexos-ulosyl)	tr	4.3 ± 0.1	–
14^{Cc}	24.60	350	577	487(10), 473(40), 413(100), 371(15), 323(27)	Methyl-luteolin 2″-*O*-deoxyhexosyl-6-*C*-hexoside	tr	1.60 ± 0.07	–
					Total phenolic acids	5.00 ± 0.06	26.2 ± 0.2	< 0.001
					Total flavonoids	6.2 ± 0.3	62 ± 1	< 0.001
					Total phenolic compounds	11.1 ± 0.3	88 ± 1	< 0.001

Phenolic compounds used for quantification: compounds 1^{Cc} and 2^{Cc} – chlorogenic acid (y = 168,823x − 161,172; R^2 = 0.9999; LOD = 0.20 μg/mL; LOQ = 0.68 μg/mL); compounds 3^{Cc} to 14^{Cc} – apigenin 6-*C*-glucoside (y = 107,025x − 61,531; R^2 = 0.9989; LOD = 0.19 μg/mL; LOQ = 0.63 μg/mL). tr – traces (below LOQ). nd – not detected (below LOD).

Table 2
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification and quantification of phenolic compounds in *A. citrodora* infusions, standard and reserve lots (mean ± SD, n = 9).

Peak	Rt (min)	λ_{max} (nm)	Pseudomolecular ion $[M-H]^-$ (m/z)	MS^2 (m/z)	Tentative identification	Quantification (μg/mL)		*t*-Students test *p*-value
						Standard	Reserve	
1^{Ac}	4.18	280	461	315(8), 135(28)	Verbascoside	3.1 ± 0.1	3.91 ± 0.10	< 0.001
2^{Ac}	12.54	344	637	351(100), 285(89)	Luteolin 7-*O*-diglucuronide	67.7 ± 0.5	87.70 ± 1.02	< 0.001
3^{Ac}	13.68	314	163	119(100)	*p*-Coumaric acid	1.65 ± 0.04	1.91 ± 0.04	< 0.001
4^{Ac}	15.44	338	621	351(100), 269(20)	Apigenin 7-*O*-diglucuronide	8.04 ± 0.06	9.99 ± 0.08	< 0.001
5^{Ac}	15.90	330	623	461(18), 315(5)	Verbascoside	73 ± 1	93 ± 1	< 0.001
6^{Ac}	17.42	350	651	351(100), 299(5)	Chrysoeriol 7-*O*-diglucuronide	12.6 ± 0.2	11.1 ± 0.1	< 0.001
7^{Ac}	18.60	330	623	461(18), 315(5)	Isoverbascoside	2.6 ± 0.01	4.4 ± 0.1	< 0.001
8^{Ac}	18.68	330	623	461(15), 315(10)	Forsythoside	2.46 ± 0.01	4.50 ± 0.03	< 0.001
9^{Ac}	18.79	350	491	315(100), 300(23)	Isorhamnetin 3-*O*-glucuronide	5.65 ± 0.03	7.70 ± 0.04	< 0.001
10^{Ac}	20.29	330	637	491(5), 461(60), 315(13)	Eukovoside	2.6 ± 0.2	3.37 ± 0.05	< 0.001
11^{Ac}	26.52	330	651	505(7), 475(22)	Martinoside	2.14 ± 0.05	2.59 ± 0.04	< 0.001
					Total caffeoyl phenylethanoid derivatives	86.1 ± 1	112 ± 2	< 0.001
					Total phenolic acids	1.65 ± 0.04	1.91 ± 0.04	< 0.001
					Total flavonoids	94.0 ± 0.8	117 ± 1	< 0.001
					Total phenolic compounds	182 ± 2	230 ± 3	< 0.001

Phenolic compounds used for quantification: compounds 1^{Ac}, 5^{Ac}, 7^{Ac}, 8^{Ac}, 10^{Ac}, and 11^{Ac} – caffeic acid (y = 388,345x − 406,369; R^2 = 0.9939; LOD = 0.78 μg/mL; LOQ = 1.97 μg/mL); compounds 2^{Ac}, 4^{Ac}, 6^{Ac}, and 9^{Ac} – apigenin 7-*O*-glucoside (y = 10,683x − 45,794; R^2 = 0.999; LOD = 0.10 μg/mL; LOQ = 0.53 μg/mL); compound 3^{Ac} – *p*-coumaric acid (y = 301,950x + 6966,7; R^2 = 0.9999; LOD = 0.68 μg/mL; LOQ = 1.61 μg/mL). Total caffeoyl phenylethanoid derivatives includes verbasoside.

respectively), as also the concentration of total phenolic compounds (241 μg/mL). All the compounds detected were coincident with those identified in a previously studied sample (Pereira, Pimenta, et al., 2017), with luteolin 7-*O*-diglucuronide and verbascoside as the major compounds found. In another study performed by our research group, a similar phenolic profile was obtained for hydromethanolic extracts of the plant, where the most abundant compounds were also common to those detected herein (Pereira, Barros, et al., 2017).

Contrarily to the results obtained for *C. citratus* and *A. citrodora*, the infusions prepared from the standard lots of *T. citriodorus* revealed slightly higher concentrations of phenolic compounds than those obtained from the reserve lots, with a total amount of 170 and 154 μg/mL, respectively. Both lots revealed a prevalence of phenolic acids (111 and 92 μg/mL for standard and reserve lots, respectively), with rosmarinic acid as the most abundant one, followed by lithospermic acid A and rosmarinic acid hexoside. Regarding flavonoids, luteolin 7-*O*-glucuronide, apigenin 7-*O*-glucuronide, eriodictyol *O*-glucuronide, and quercetin *O*-glucuronide were the major molecules identified. The compounds detected in these samples have been previously found and identified by our research group in *Thymus vulgaris* L. infusions, decoctions, hydroalcoholic, and methanolic extracts (Martins et al., 2015; Pereira, Barros, et al., 2016; Pereira, Pimenta, et al., 2016). In general, luteolin 7-*O*-glucuronide and rosmarinic acid were the most abundant compounds in the different studied extracts, as in the standard and

Table 3
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification and quantification of phenolic compounds in *T. citriodorus* infusions, standard and reserve lots (mean ± SD, n = 9).

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (m/z)	MS^2 (m/z)	Tentative identification	Quantification (μg/mL)		*t*-Students test *p*-value
						Standard	Reserve	
1^{Tc}	6.24	320	341	179(100), 135(88)	Caffeic acid hexoside	2.09 ± 0.04	2.69 ± 0.04	< 0.001
2^{Tc}	6.38	282, 327	611	449(100), 287(14)	Eriodictyol *O*-dihexoside	tr	tr	–
3^{Tc}	9.13	338	593	473(20), 383(33), 353(27), 297(5)	Apigenin 6,8-di-*C*-glucoside	tr	1.05 ± 0.06	–
4^{Tc}	14.50	340	477	301(100)	Quercetin *O*-glucuronide	5.68 ± 0.02	5.65 ± 0.03	0.242
5^{Tc}	15.33	285, 331	463	287(100)	Eriodictyol *O*-glucuronide	6.17 ± 0.09	5.3 ± 0.1	< 0.001
6^{Tc}	16.60	322	521	359(100), 197(13), 179(36), 161(62), 135(21)	Rosmarinic acid hexoside	11.7 ± 0.3	4.24 ± 0.06	< 0.001
7^{Tc}	17.74	348	461	285(100)	Luteolin 7-*O*-glucuronide	39 ± 1	41.2 ± 0.9	0.018
8^{Tc}	20.85	330	359	197(17), 179(35), 161(100), 135(29)	Rosmarinic acid	78 ± 2	66 ± 2	< 0.001
9^{Tc}	21.99	336	445	269(100)	Apigenin 7-*O*-glucuronide	7.53 ± 0.01	9.2 ± 0.2	< 0.001
10^{Tc}	24.25	290, 326sh	537	493(50), 359(17), 295(33), 179(75), 135(100)	Lithospermic acid A	19.4 ± 0.6	18.8 ± 0.7	0.138
					Total phenolic acids	111 ± 3	92 ± 2	< 0.001
					Total flavonoids	58 ± 1	62 ± 1	0.008
					Total phenolic compounds	170 ± 4	154 ± 4	0.002

Phenolic compounds used for quantification: compound 1^{Tc} – chlorogenic acid (y = 168,823x − 161,172; R^2 = 0.9999; LOD = 0.20 μg/mL; LOQ = 0.68 μg/mL); compounds 2^{Tc} and 5^{Tc} – naringenin (y = 18,433x + 78,903; R^2 = 0.9998; LOD = 0.17 μg/mL; LOQ = 0.81 μg/mL); compound 3^{Tc} – apigenin 6-*C*-glucoside (y = 107,025x − 61,531; R^2 = 0.9989; LOD = 0.19 μg/mL; LOQ = 0.63 μg/mL); compound 4^{Tc} – quercetin 3-*O*-glucoside (y = 34,843x − 160,173; R^2 = 0.9998; LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL); compounds 6^{Tc}, 8^{Tc}, and 10^{Tc} – rosmarinic acid (y = 191,291x − 652,903; R^2 = 0.999; LOD = 0.15 μg/mL; LOQ = 0.68 μg/mL); compounds 7^{Tc} and 9^{Tc} – apigenin 7-*O*-glucoside (y = 10,683x − 45794; R^2 = 0.999; LOD = 0.10 μg/mL; LOQ = 0.53 μg/mL). tr – traces (below LOQ).

Table 4
Antioxidant activity of *C. citratus*, *A. citriodira*, and *T. citriodorus* standard and reserve lots infusions, expressed as EC_{50} values (mg/mL, mean ± SD, n = 9).

	Reducing power	Radical scavenging activity		Lipid peroxidation inhibition
	Ferricyanide/Prussian blue	DPPH scavenging activity	β-carotene/linoleate	TBARS
C. citratus standard lot	2.33 ± 0.05	3.49 ± 0.03	3.66 ± 0.09	0.75 ± 0.03
C. citratus reserve lot	1.01 ± 0.01	1.43 ± 0.06	2.01 ± 0.03	0.37 ± 0.01
Student's *t*-test *p*-value	< 0.001	< 0.001	< 0.001	< 0.001
A. citrodora standard lot	0.205 ± 0.001	0.39 ± 0.05	0.93 ± 0.02	0.082 ± 0.001
A. citrodora reserve lot	0.169 ± 0.001	0.25 ± 0.01	0.92 ± 0.01	0.088 ± 0.001
Student's *t*-test *p*-value	< 0.001	0.013	0.350	0.002
T. citriodorus standard lot	0.228 ± 0.003	0.34 ± 0.01	1.01 ± 0.06	0.118 ± 0.001
T. citriodorus reserve lot	0.393 ± 0.002	0.32 ± 0.02	0.73 ± 0.03	0.117 ± 0.001
Student's *t*-test *p*-value	< 0.001	0.251	0.035	0.112

Trolox EC_{50} values: 41 μg/mL (reducing power), 42 μg/mL (DPPH scavenging activity), 18 μg/mL (β-carotene bleaching inhibition) and 23 μg/mL (TBARS inhibition).

reserve lots of *T. citriodorus* assessed in the present work.

3.2. Antioxidant activity

The antioxidant activity results obtained for the standard and reserve lots of *C. citratus*, *A. citrodora*, and *T. citriodorus*, expressed as EC_{50} values, are present in Table 4. *C. citratus* reserve lot (0.37–2.01 mg/mL) revealed a higher antioxidant activity than the standard lot (0.75–3.66 mg/mL), with lower EC_{50} values in the four assays performed, which is in accordance with its higher phenolic compounds concentration.

Regarding *A. citrodora* lots, the differences between the results obtained in the four assays were not statistically significant, with the exception of the reducing power, where the reserve lot exhibited better results (EC_{50} values of 0.169 and 0.205 mg/mL for the reserve and the standard lots, respectively).

Similarly, for *T. citriodorus* infusions, the standard and reserve lots gave similar results of antioxidant capacity, except for the reducing power assay, but with the standard lot revealing the highest activity (EC_{50} values of 0.228 and 0.393 mg/mL for the standard and reserve lots, respectively), which could be explained by its higher concentration of phenolic compounds.

All the EC_{50} values obtained for the infusions were higher than those of the positive control (trolox), nevertheless, these results were obtained for the infusions prepared according to the label specifications and, despite their high concentration value, the EC_{50} values were all lower than the recommended concentration (3 mg/mL). As the only exception, the standard lot of *C. citratus* presented EC_{50} values slightly higher than the recommended concentration in the DPPH scavenging activity (3.49 mg/mL) and β-carotene bleaching inhibition (3.66 mg/mL) assays.

Correlation factors between the antioxidant activity and the phenolic composition were obtained, using a Pearson's correlation analysis, after verifying the normality through a Shapiro-Wilk test. Regarding *C. citratus* infusions, higher statistically significant correlations were found between the total phenolic compounds amount and the EC_{50} values obtained in DPPH scavenging activity ($R^2 = -0.886$, p-values = 0.019) and reducing power ($R^2 = -0.999$, p-values ≤ 0.001) assays; otherwise, lower correlations were obtained for β-carotene bleaching inhibition ($R^2 = -0.600$, p-values = 0.208) and lipid peroxidation inhibition by TBARS assays ($R^2 = -0.771$, p-values = 0.072). The antioxidant capacity of *A. citrodora* infusions was significantly correlated to the concentration of total phenolic compounds regarding DPPH scavenging activity ($R^2 = -0.892$, p-values = 0.017), reducing power ($R^2 = -0.997$, p-values < 0.001), and TBARS assays ($R^2 = -0.946$, p-values = 0.004), contrarily to the β-carotene bleaching inhibition test ($R^2 = -0.475$, p-values = 0.341). In what concerns *T. citriodorus* infusions, the total concentrations of phenolic compounds were highly correlated to the reducing power ($R^2 = -0.959$, p-values = 0.003) and β-carotene bleaching inhibition ($R^2 = -0.880$, p-values = 0.021) assays, while for the TBARS assays ($R^2 = -0.650$, p-values = 0.162), lower correlation factors were obtained. The DPPH scavenging activity ($R^2 = -0.422$, p-values = 0.405) was not correlated with the phenolic compounds, meaning that other compounds (e.g. triterpenoids, vitamins or other organic compounds) might be related to the scavenging effect revealed by this species.

4. Conclusion

In general, the samples presenting the highest concentration of phenolic compounds also revealed the highest antioxidant activity. The infusions prepared from reserve lots of *C. citratus* and *A. citrodora* revealed higher amounts of total phenolic acids and flavonoids than those obtained from standard lots. In the case of *T. citriodorus* infusions, the standard and reserve lots did not reveal significant differences regarding the total flavonoids and total phenolic compounds concentration. The results obtained in this study will allow consumers to make a conscientious choice between the two categories (standard and reserve lots), not only based on their sensory characteristics, but also on their bioactive potential.

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